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ACCELERATED INDUCTION OF HEPATOMAS IN FAST NEUTRON IRRADIATED MICE INJECTED WITH CARBON TETRACHLORIDE

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ABSTRACT

Young adult (C57L x A)F_1 hybrid mice received a single whole body exposure to fission spectrum fast neutrons (165-306 rad). Subgroups of these mice then received a single subcutaneous injection of CCl_4, given either at 2, 12, 15, or 18 months post-irradiation. Control non-irradiated mice received a single injection of CCl_4. Other groups of mice were exposed to a single 500 rad dose of 250 KVP X-rays. The incidence of hepatomas was markedly increased in the neutron-irradiated mice (19%) as compared with that in the X-irradiated mice (2%). In the neutron-irradiated mice injected with CCl_4, the hepatoma incidence attained a value of 61%, three times that in the mice irradiated with neutrons only. In addition, marked pleomorphism and atypicality of liver cell nuclei was evident in almost all of the neutron-CCl_4 mice, but was observed in only 6 of the 47 mice exposed to neutrons only. Of the small group of mice which received CCl_4 but no irradiation, and sacrificed up to 22 months later, none exhibited hepatomas or nuclear abnormalities. In this system therefore, CCl_4 seems to act as a promoting agent in liver carcinogenesis. These findings, taken together with other data in the literature, support the concept that hepatoma induction is accelerated as a consequence of the action of a specific proliferative stimulus on cells bearing a latent radiation-induced alteration. The general question of the role of proliferative stimuli in radiation carcinogenesis is discussed.

SUMMARY

The Problem:

Although it is well established that ionizing radiation is a carcinogenic agent in animals and man, there is still a lack of information as to the relative potency of high LET radiations (such as fast neutrons) versus low LET radiations (such as X-radiation) with respect to long-term carcinogenic hazard. Furthermore, in the light of the accumulating evidence that the development of tumors involves at least two separate phases, i.e., so-called "initiation," and "promotion," it is of some importance to gain knowledge on the role of promoting agents in radiation carcinogenesis. In the system investigated here, carbon tetrachloride (CCl_4) was used to induce mitotic activity in the liver after irradiation.[4] The hypothesis tested was whether the application of this specific proliferative stimulus would act as a promoting agent for tumor formation in the target tissue, in this case liver, under these conditions.

The Findings:

Young adult (C57L x A)F_1 hybrid mice received a single whole body exposure to fission spectrum fast neutrons (165–306 rad). Subgroups of these mice then received a single subcutaneous injection of CCl_4, given either at 2, 12, 15, or 18 months post-irradiation. Control non-irradiated mice received a single injection of CCl_4. Other groups of mice were exposed to a single 500 rad dose of 250 KVP X-rays. The incidence of hepatomas was markedly increased in the neutron-irradiated mice (19%) as compared with that in the X-irradiated mice (2%). In the neutron-irradiated mice injected with CCl_4, the hepatoma incidence attained a value of 61%, three times that in the mice irradiated with neutrons only. In addition, marked pleomorphism and atypicality of liver cell nuclei was evident in almost all of the neutron-CCl_4 mice, but was observed in only 6 of the 47 mice exposed to neutrons only. Of the small group of mice which received CCl_4 but no irradiation, and sacrificed up to 22 months later, none exhibited hepatomas or nuclear abnormalities. In this system therefore, CCl_4 seems to act as a promoting agent in liver carcinogenesis. These findings, taken together with other data in the literature, support the concept that hepatoma induction is accelerated as a consequence of the action of a specific proliferative stimulus on

cells bearing a latent radiation-induced alteration. The general question of the role of proliferative stimuli in radiation carcinogenesis is discussed.

INTRODUCTION

It has been generally accepted that carcinogenesis is a multi-stage process, involving at least two separate phases, i.e., an "initiating action", and a "promoting phase" (1). With respect to carcinogenesis by ionizing radiations, it is becoming increasingly more evident that the physiological, hormonal, and local tissue events implicated in the final manifestation of many neoplasms are in fact specific proliferative stimuli or growth regulators for target tissues, and therefore can be considered as promoting factors acting on radiation-altered cells (2,3). The experimental studies of Furth et al (4) on radiation-induced mammatropic tumors, and those of Cronkite et al (5) on mammary tumors provide excellent examples of this concept.

However, our knowledge of the nature of the "initiating" action of ionizing radiation is very limited. The investigation of carcinogenesis following exposure to high LET versus low LET radiations appears to offer some possible new insights into this problem, particularly in view of recent developments in karyotype analysis of tumor and irradiated cells (6), and of the application of cloning techniques for investigating radiation effects at the cellular level(7).

In previously published studies (8,9,10) on fast neutron carcinogenesis in mice we observed a large increase in the incidence of gastrointestinal neoplasms in LAF_1 mice exposed to a single high sublethal dose of fast neutrons, whereas the occurrence of such neoplasms was much less frequent in mice exposed to 250 KVP X-rays. The incidence of neoplastic lesions in the kidneys of the neutron-irradiated mice was only slightly increased over that of non-irradiated controls, while hepatomas were not more frequent after irradiation. The question arises whether the difference in carcinogenic response of the gastrointestinal epithelium versus liver or kidney with respect to fast neutrons, may be related to the fact that the latter tissues exhibit a low mitotic rate and cell turnover, whereas gastrointestinal epithelium is a rapidly proliferating tissue. On the supposition that ionizing radiation initiates a latent pre-neoplastic, hereditable change of a more or less permanent nature (2,11) in the cells of liver and kidney, as well as in the gastrointestinal epithelium, it was of interest to establish whether the tumorigenic effect of fast neutron radiation could be more readily evoked in the

liver or kidneys, by applying a specific mitotic stimulus to these otherwise non-proliferating tissues.

The persistence of latent radiation-induced mitotic aberrations in the resting liver for periods of several months after irradiation is well established. These cytological changes become overt when the liver is induced to proliferate by administration of CCl_4 (12) or by partial hepatectomy (13,14). Similar radiation-induced latent injury to proliferative capacity has been observed also in the kidneys (15).

MATERIALS AND METHODS

The mice employed were (C57L x A)F_1 hybrids (so-called LAF_1) of both sexes. At age 10-15 weeks old, the mice received a single whole body exposure to fast neutrons (with a spectrum similar to that of fission neutrons), produced in the 60-inch cyclotron at the Crocker Laboratory of the University of California, Berkeley by the $Be(p,n)^{9}B$ reaction. The irradiation procedure has been described previously (8); and the physical characteristics of these neutrons, as well as the details of dosimetry have been reported by Tochlin et al (16). The dose rate was approximately 30 rad per minute. The neutron doses employed were in the range from 165 rad to 306 rad. Other groups of LAF_1 mice 10-15 weeks old, were exposed to a single sublethal dose of 250 KVP X rays (500 rad) at dose rate of approximately 28 rad per minute. Subgroups of these irradiated mice then received a single subcutaneous injection of 0.15 ml of 40% CCl_4 in sesame oil, given either at 2, 12, 15 or 18 months post-irradiation. Control non-irradiated mice received a single injection of CCl_4. Tissues were taken from mice which died or were sacrificed up to 28 months post-irradiation. The tissues were fixed in formalin, and stained with hematoxylin and eosin after routine processing.

RESULTS

Hepatomas: A summary of the findings is given in Table I. Of 58 neutron-irradiated mice examined to date, which did not receive CCl_4, 11 (19%) showed hepatomas. By comparison, in 42 mice exposed to 500 rad of X-rays, only 1 hepatoma was found (2%). Thus, the hepatoma incidence in the neutron-irradiated mice is markedly increased over that of the X-irradiated group. In the neutron-irradiated mice injected with CCl_4, the hepatoma incidence attained a level of 61% (11 out of 18), three times that found in the mice which were exposed to fast neutrons only.

TABLE I

INCREASED INCIDENCE OF HEPATOMAS IN IRRADIATED MICE RECEIVING A SINGLE INJECTION OF CARBON TETRACHLORIDE

TREATMENT	AGE AT DEATH (mos)	NO. OF MICE	HEPATOMAS No.	HEPATOMAS %
Neutrons (165-306 rad)	20-30	58	11	19
Neutrons + CCl_4 (171-306 rad)	20-31	18	11	61
X-rays (500 rad)	18-33	41	1	2
CCl_4 only (0 rad)	17-22	6	0	0

The tumors varied in size from 5 mm to several cm. diameter(Figure 1) the largest tumors being in the neutron-CCl_4 mice. Histologically, the tumors in all groups were similarly composed of cords and nests of relatively well-differentiated hepatic cells (Figure 2). They were generally well-circumscribed, and metastases were not observed. In addition, of the 7 mice in the neutron-CCl_4 group which did not have hepatomas, 5 showed marked pleomorphism and atypicality of liver cell nuclei (Figure 3). Similar, but generally less severe nuclear changes were observed in only 6 of the 47 mice, irradiated with neutrons only, which did not have hepatomas. Finally, of the small group of mice which received CCl_4 but no irradiation, and were sacrificed up to 22 months later, none exhibited hepatomas or nuclear abnormalities.

Other tumors: The incidence of tumors other than hepatomas was comparable, in the various groups, to that observed in previous reports by us (10), and by Upton et al (17).

DISCUSSION

Although the number of animals involved in these experiments is relatively small, certain conclusions appear to be warranted from the data. First, that fast neutron radiation may have a relatively greater potency than X-rays in eliciting hepatomas in these mice. This observation is at variance with earlier studies by ourselves (10) and by Upton et al (17) in which fast neutron irradiation and X-irradiation resulted in similar hepatoma incidence (2-3%). The marked increase in incidence in the present series may be due, in part, to the more careful examination of the livers. However, further data are obviously required. It is of interest that Rosen et al (18) have recently reported a relative potency between 2 and 3 for fission-spectrum neutrons versus X-rays for the production of renal neoplasms in rats.

Second, a single inoculation of CCl_4 to neutron-irradiated mice resulted in a three-fold increase in hepatoma incidence. Although the incidence of hepatomas in non-irradiated mice can be greatly increased by multiple feedings of CCl_4, as reported by Edwards (19), a single injection of CCl_4 did not result in hepatoma formation (19). The absence of liver neoplasms in our small non-irradiated group which received CCl_4 alone, is in accord with this observation.

It appears, therefore, that in this system CCl_4 acts as a promoting agent for liver carcinogenesis. The proliferative response of the liver to this hepatotoxic agent is well known. However, this proliferative effect may not be the sole basis for the promoting action of CCl_4. Ingle and Baker (20) carried out multiple (12 times) partial hepatectomies

Fig. 1 Gross view of hepatoma in neutron irradiated-CCl_4 mouse. The tumor mass occupies most of the liver.

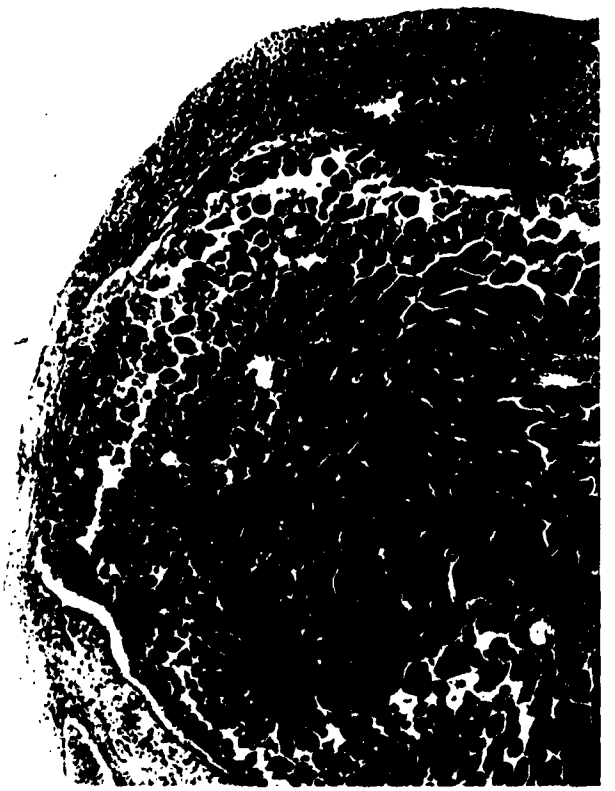

Fig. 2 Low power view of well-circumscribed hepatoma from 28 month old LAF_1 mouse irradiated with fast neutrons (300 rad) at age 3 months, followed by a single injection of CCl_4 12 months later. (x 35)

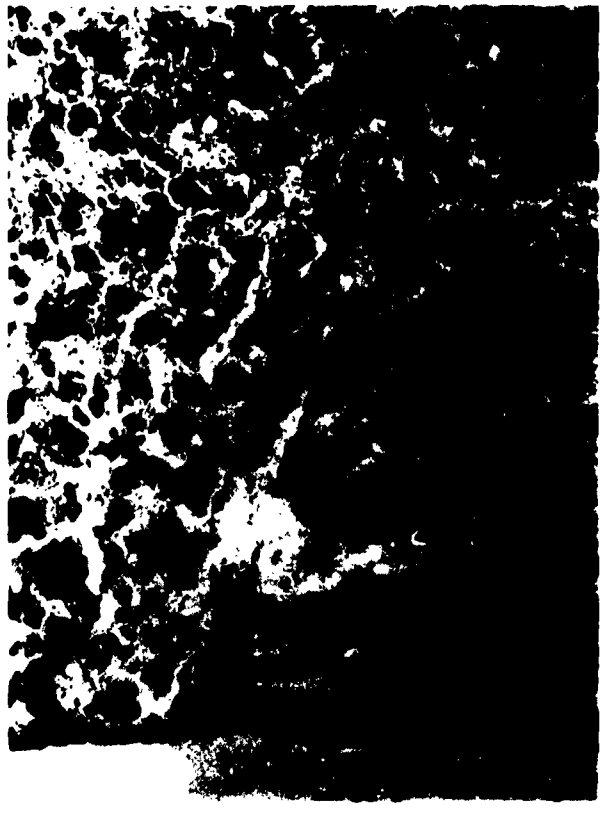

Fig. 3 High power view showing nuclear variability in liver cells of another mouse similarly treated. This mouse did not have a hepatoma. (x 250)

in non-irradiated rats during a period of 1 year. Under these conditions of repetitive hyperplasia, no development of liver neoplasia was found. Since repeated feeding of CCl_4 does result in hepatomas in non-irradiated mice (19), it is possible that the promoting action of CCl_4 in the present experiments involves factors in addition to a proliferative stimulus. Pertinent to this is the recent finding of Smuckler et al (21) of an early intracellular defect in protein synthesis associated with alterations in the endoplasmic reticulum in the livers of rats, following a single intubation with CCl_4.

There is considerable evidence that high LET radiation produces greater acute damage to cells, when compared with low LET radiations. In animal experiments, this is reflected in the greater histological damage, and the relatively slower regeneration of the intestinal epithelium (22) and of the bone marrow (23) after exposure of mice to fast neutrons. At the cellular level, Barendsen (24) has shown that the surviving fraction of cultured human kidney cells irradiated in vitro with α-radiation, decreases exponentially as dose increases. No shoulder was evident in the curve on a semi-logarithmic plot. The LD_{37} was only 65 rads. The α-irradiated cells exhibited no repair on dose fractionation. These data are interpreted by Berendsen as a "single event" type of action, by this high LET radiation. By contrast, the survival curves for such cells after exposure to low LET radiation (200 KV X-rays) exhibit a distinct shoulder, and show recovery when the radiation dose is fractionated (25); (cf. also 26). Such effects are characteristic of the "cumulative" type of action of low LET radiation, according to Barendsen. Similar results have been obtained by Andrews and Berry (27, 28) in studying the effect of fission-spectrum fast neutron radiation on reproductive capacity of mouse lymphocytic leukemia cells, measured by means of transplantability. The response of these cells to fast neutron irradiation, according to these authors, is a "no-threshold, no-recovery one".

These differences in the mode of action of high LET versus low LET radiations, reflected in the relatively more severe intracellular defect, probably at the chromosomal level (cf.7), in cells exposed to high LET radiations, may well be the basis for the marked difference in carcinogenic potency between fast neutrons and X-rays, as observed in the liver and in the gastrointestinal epithelium. According to this concept, carcinogenic "initiation" is correlated with the degree and persistence of a radiation-induced cellular defect or alteration within a population of cells. The fact that fast-neutron irradiated cells show the "single event", no-recovery type of response with respect to reproductive capacity, as discussed above implies that any radiation-induced genetic damage incurred by these cells would persist for more prolonged periods without repair, as compared with X-irradiated cells. Although the true nature

of this intracellular lesion is unknown, it would appear to reside at the chromosomal or subchromosomal level, and to be associated with an increased genetic instability or mutability in this population; as a consequence, the eventual appearance of a small number of the postulated "mutant" neoplastic cells becomes statistically more probable. The establishment of such "mutant" cell clones and their emergence as an actual tumor mass, presumably by selective proliferation, would depend, at least in part, upon the level of proliferative activity in the specific tissue involved. Under proper conditions (e.g. mitotic stimulation by CCl_4 damage) these aberrant cells and their progeny would be more and more likely to change in the direction of release from hormonal or other regulatory control (cf. 29). Similar accelerated induction of renal neoplasms has been shown to occur in X-irradiated mice following the proliferative stimulus of unilateral nephrectomy (30).

What is the promoting agent in the case of the neutron-irradiated mice which did not receive CCl_4? One would have to postulate here that the cell turnover in the liver although extremely slow, would, over a period of 2 years or more, permit the eventual emergence of the radiation-initiated neoplastic cell clones. Any endogenous factors (for example, hormonal) or extrinsic influences (for example, dietary or toxic) which increase cell turnover in the liver, would then be expected to hasten the carcinogenic process under these conditions.

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(Over)

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2. Fast neutrons - Biological effects.
3. Liver - Mitosis.

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IV. MR005.08-5200.

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